CLAIMS

1. A strand-specific polynucleotide nickase comprising an endonuclease which comprises a first subunit and a second subunit and which recognises an asymmetric nucleotide recognition sequence, wherein the first subunit comprises a catalytic domain capable of cleaving one strand of a DNA duplex, and the second subunit is incapable of cleaving the other strand of the DNA duplex.

2. A nickase according to claim 1, wherein the second subunit comprises an inactivated endonuclease catalytic domain.

3. A nickase according to claim 1, wherein the catalytic domain is incapable of cleaving one strand of the DNA duplex in the absence of the second subunit.

4. A nickase according to claim 1, wherein the recognition sequence comprises 4 or more nucleotides.

5. A nickase according to claim 1, wherein the catalytic domain is capable of cleaving one strand of the DNA duplex within the recognition sequence.

6. A nickase according to claim 1, wherein the first subunit comprises a subunit from a heteromeric restriction endonuclease.

7. A nickase according to claim 1, wherein the second subunit comprises a subunit from a heteromeric restriction endonuclease modified to render inactive the catalytic domain thereof.

8. A strand-specific polynucleotide nickase comprising a heteromeric restriction endonuclease which comprises a first subunit and a second subunit and which recognises an asymmetric nucleotide recognition sequence, wherein the first subunit comprises a catalytic

domain capable of cleaving one strand of a DNA duplex, and the second subunit is modified to render the catalytic domain thereof inactive.

9. A nickase according to claim 8, wherein the heteromeric restriction endonuclease comprises R.*Bpu*10I.

10. A process for producing a strand-specific polynucleotide nickase, which process comprises inactivating the catalytic activity of one subunit of a restriction endonuclease, wherein the endonuclease comprises a first subunit comprising a catalytic domain capable of cleaving one strand of a DNA duplex and a second subunit comprising a catalytic domain capable of cleaving the other strand of the DNA duplex, and the endonuclease recognises an asymmetric nucleotide recognition sequence.

11. A process according to claim 10, wherein the recognition sequence comprises 4 or more nucleotides.

12. A process according to claim 10, wherein the endonuclease is capable of cleaving at least one strand of the DNA duplex within the recognition sequence.

13. A process according to claim 10, wherein the endonuclease comprises a heteromeric restriction endonuclease.

14. A process according to claim 13, wherein the endonuclease comprises R.*Bpu*10I.

15. A process according to claim 10, wherein the step of inactivating the catalytic activity of one subunit of the restriction endonuclease comprises non-specific mutagenesis of the subunit.

16. A process according to claim 10, wherein the step of inactivating the catalytic activity 10of one subunit of the restriction endonuclease comprises identifying the catalytic domain of the

subunit and subsequently introducing mutations into the catalytic domain by site-specific mutagenesis.

17. A process according to claim 16, wherein the catalytic domain is identified by comparing the protein sequence of the subunit with the protein sequence motifs from other restriction endonucleases.

18. A strand-specific polynucleotide nickase obtainable by a process as defined in any of claims 10 to 17.

19. A method for introducing one or more site-specific nicks into pre-selected strands of a DNA duplex, which comprises contacting the DNA duplex with a nickase as defined in claim 1 under conditions to permit nickase activity.

20. A method according to claim 19 wherein the DNA duplex comprises circular double-stranded DNA, and the one or more site–specific nicks produce circular single-stranded DNA.

21. A method according to claim 19 which comprises production of nested deletions in a DNA molecule.

22. A method according to claim 19 wherein the one or more site-specific nicks produce a vector for use in a ligation-independent cloning method.

23. Use according to claim 19 wherein the one or more site-specific nicks produce a covalently closed linear DNA molecule.

24. A kit for producing one or more site-specific nicks in pre-selected strands of a DNA duplex, comprising a first nickase as defined in claim 1 and a second nickase as defined in claim 1, wherein the first nickase and the second nickase recognise the same recognition sequence, the

first nickase is capable of cleaving a first strand of the DNA duplex and the second nickase is capable of cleaving a second strand of the DNA duplex.

25. A kit according to claim 24, wherein the first and second subunits of the first and second nickases comprise subunits from a single heteromeric restriction endonuclease, wherein the first nickase comprises a first subunit capable of cleaving the first strand of the DNA duplex and a second subunit comprising a catalytic domain inactivated to be incapable of cleaving the second strand of the DNA duplex, and wherein the second nickase comprises a first subunit capable of cleaving the second strand of the DNA duplex and a second subunit comprising a catalytic domain inactivated to be incapable of cleaving the first strand of the DNA duplex.

26. A kit according to claim 24, for producing circular single-stranded DNA from circular double-stranded DNA, which kit further comprises an exonuclease.

27. A kit according to claim 26, wherein the kit further comprises a circular double-stranded DNA molecule comprising the recognition sequence recognised by the first and second nickases.

28. A kit for use in a cloning method comprising a nickase as defined in claim 1, and a vector comprising a recognition sequence for a restriction endonuclease flanked on each side by the recognition sequence of the nickase, wherein the recognition sequences of the nickase are inverted with respect to each other such that the nickase is capable of cleaving different strands of the vector on each side of the recognition sequence for the restriction endonuclease.

29. A kit for producing covalently closed linear DNA, comprising a nickase as defined in claim 1, and a vector comprising a recognition sequence for a restriction endonuclease flanked on each side by a pair of recognition sequences of the nickase, wherein the recognition sequences of each pair are inverted with respect to each other such that the nickase is capable of cleaving each strand of the vector on each side of the recognition sequence for the restriction endonuclease, and

wherein one strand of the sequence between each pair of recognition sequences comprises a self-complementary sequence capable of forming a hairpin loop.